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Electrochemical removal of biofilms from titanium dental implant surfaces

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ABSTRACT

The infection of dental implants may cause severe inflammation of tissue and even bone degradation if not treated. For titanium implants, a new, minimally invasive approach is the electrochemical removal of the biofilms including the disinfection of the metal surface. In this project, several parameters, such as electrode potentials and electrolyte compositions, were varied to understand the underlying mechanisms. Optimal electrolytes contained iodide as well as lactic acid. Electrochemical experiments, such as cyclic voltammetry or measurements of open circuit potentials, were performed in different cell set-ups to distinguish between different possible reactions. At the applied potentials of $E < -1.4$ V, the hydrogen evolution reaction dominated at the implant surface, effectively lifting off the bacterial films. In addition, several disinfecting species are formed at the anode, such as triiodide and hydrogen peroxide. *Ex situ* tests with model biofilms of *E. coli* clearly demonstrated the effectiveness of the respective anolytes in killing the bacteria, as determined by the LIVE/DEAD™ assay.
Using optimized electrolysis parameters of 30 s at 7.0 V and 300 mA, a 14-day old wildtype biofilm could be completely removed from dental implants *in vitro*.

1. Introduction

The success of dental implants as replacement for missing teeth is lowered by the complications of peri-implant mucositis and peri-implantitis [1,2]. Mucositis is defined as the reversible inflammation of soft tissue surrounding dental implants, whereas peri-implantitis is often described as irreversible inflammatory process that degenerates connective tissue between bone and osseointegrated oral implants and is often followed by the resorption of surrounding bone. Such severe complications are caused by the colonization of different pathogenic bacteria on the implant surface and their organization in bacterial biofilms. In case of medical malpractice or omitted treatment peri-implantitis can lead to complete disintegration of the implant [3,4]. Recent methods to treat peri-implantitis [5,6,7] include mechanical decontamination and local antiseptic or antibiotic treatment. Implant surface treatments are scaling, laser decontamination and photo-dynamic therapy, powder-blasting with biocompatible abrasives, chlorhexidine or hydrogen peroxide irrigation, or local application of antibiotics [5,6,7]. Most of these debridement protocols for dental implants have been derived from periodontology.

A novel, minimally invasive approach [8,9,10] to remove and disinfect dental implants utilizes the fact that titanium is an electrically conducting metal and the number of adherent microorganisms on dental implants could be drastically reduced by electrolysis [11,12,13]. In numerous case studies to verify the mode of action [14,15], the use of chlorhexidine [16,17], citric acid [18], hydrogen peroxide [18], iodine compounds [19,20,21,22], variation of the pH value [23], gas bubbles [24], powder-blasting and mechanical debridement [25,26] to remove biofilms from implant surfaces, were investigated. Chemical disinfectants [27,28] or antibiotics affect mainly the permeation properties of the cytoplasmic and external membranes of bacteria, *e.g.* by interactions with phospholipids or by denaturation of specific proteins, by oxidation of peptide links and structurally important compounds and, in the case of iodine compounds, by blocking electron transport through electrophilic reactions with enzymes of the respiratory chain of aerobic microorganisms [15]. The *in situ* generation of disinfectants is well-known for water disinfection [29,30,31] and was successfully transferred to dental implant purification in recent proof-of-concept studies [11–13,28,32–45] and by exploitation of the bioelectrical effect [46,47,48]. Electrochemical treatment of dental implants combines the antibacterial efficacy of bactericides, but with low free concentration of toxic substances as they are only generated during electrolysis, with the direct oxidation of bacterial enzymes and proteins. Especially results from D. Ren et al. [49,50] demonstrated that pathogenic bacteria such as *Streptococcus mutans*, *Staphylococcus aureus* and *Pseudomonas*

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aeruginosa could be efficiently killed by low level direct current treatment and/or in combination with antibiotics.

In this work we investigate the beneficial effect of electrochemical removal of *E. coli* biofilms by the hydrogen evolution reaction (HER) at titanium surfaces in combination with the *in situ* generation of a disinfecting agent. Stable and effective electrolysis parameters that fulfill typical application specifications were identified [8,9,10]. The electrolysis products were analyzed and quantified if applicable. The electrochemistry of bare titanium surfaces in the used electrolytes was investigated by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and monitoring of the open circuit potential, E_{OCP}, before and the open cell voltage, E_{CVE}, during electrolysis. In order to test the disinfecting efficacy of the method, *E. coli* biofilms were grown on different titanium surfaces and their removal under various conditions was tested by the LIVE/DEAD™ assay *via* fluorescence microscopy and monitored by infrared absorption-reflection spectroscopy (IRRAS). The morphologies of bare and modified titanium surfaces were assessed with atomic force microscopy (AFM) and scanning electron microscopy (SEM). Finally the developed method was used to remove a mature wildtype biofilm completely from a commercial dental implant.

2. Materials and methods

2.1. Chemicals and electrolytes

All chemicals were of analytical/Ph. Eur. grade and were used as received (Sigma Aldrich, Merck, Alfa Aesar). Table 1 shows the concentration levels of electrolytes that were used. All solutions were prepared from MilliQ water (Millipore) in measuring flasks.

2.2. Substrate preparation

Titanium substrates were fabricated using a conventional multipocket electron beam evaporation chamber (Pfeiffer Vacuum 50 Classic) with a quartz crystal microbalance (QCM) thickness monitor and Meissner cooling trap. Si(100) wafers (Active Business Company) were placed on the sample holder disc and mounted 20 cm above the source at the same level as the QCM element. Evaporation was conducted at pressures below 10^{-5} hPa, while the sample holder was rotated. A layer of 50 nm of titanium (99.999%, Alfa Aesar) was evaporated with a rate of 0.5 Å/s onto the wafers. Substrates were removed after allowing the system to cool down to ambient temperature and cut into 10×10 mm^2. For surface modification the substrates were stirred in concentrated HCl at RT overnight.

Table 1
Concentration levels of the investigated electrolytes; final volume 100.00 mL. KI – potassium iodide, LA – D/L lactic acid.

Solution	Chemicals	M/g mol^{-1}	m/g	n/mmol	c/mmol L^{-1}
A	Potassium iodide (KI)	166.00	12.5	75.4	754
A*	KI	166.00	12.5	75.4	754
	D/L Lactic acid (LA)	90.08	0.57	6.3	63
B	KI	166.00	6.26	37.7	377
B*	KI	166.00	6.26	37.7	377
	LA	90.08	0.29	3.2	32
C	KI	166.00	12.5	75.4	754
	L-Malic acid	134.09	0.58	4.3	43
D	KI	166.00	12.5	75.4	754
	Ascorbic acid	176.13	0.86	4.9	49
E	KI	166.00	12.5	75.4	754
	Citric acid	192.13	1.21	6.3	63
F	Sodium formate	68.01	25.0	368	$3.68 \cdot 10^3$
	LA	90.08	1.26	13.9	139
G	Sodium acetate	82.03	25.0	305	$3.05 \cdot 10^3$
	LA	90.08	1.26	13.9	139

2.3. Surface morphology characterization

Samples for AFM and SEM were immersed in 25% glutaraldehyde overnight, afterwards dipped ten times in 50% EtOH [51] and dried in a vacuum desiccator for at least 2 h. Surface topography was acquired on an atomic force microscope NT-MDT Solver Pro (NT-MDT) in semi-contact mode with a silicon tip (HA_NC Etalon, spring constant 3.5 N/m) and at different scanning areas at a scan rate of 1 Hz. The experiments were conducted in air and at room temperature. Calibration and testing of tip quality was achieved after each run by comparison with TGS1 and TGT1 test patterns (NT-MDT). Morphology of the samples was studied using a high-resolution scanning electron microscope (Atomica Amray 1920ECO) at an acceleration voltage of 10–15 kV. Samples were sputtered with ~10 nm of gold when necessary (Edwards Sputter Coater).

2.4. Infrared reflection-absorption spectroscopy

Acquisition of IR spectra of biofilms on Ti substrates was performed with a Thermo Fisher Scientific Nicolet 6700 FT-IR spectrometer (HeNe laser, λ: 632.8 nm; IRRAS unit SmartSAGA, using *p*-polarized IR radiation, incidence angle of 80° relative to the sample surface normal) with a LN-cooled mercury-cadmium-telluride detector. The beam path of the spectrometer was purged with dried and CO_2-free air. For each spectrum at least 4×256 scans were recorded from 650 cm^{-1} to 4000 cm^{-1} with a resolution of 4 cm^{-1}, followed by a baseline correction and averaging afterwards. Background was a blank substrate. The spectra were evaluated with the Thermo Fisher Omnic software (version 8.0.380).

2.5. Electrolysis set-up and cell voltage

For electrolysis four custom-built cells were used, shown in Fig. S1. Tests were conducted in all cell types. The electrodes for electrolysis were a titanium dental implant (Straumann BL Ø 4.1 mm, RC SLA™, Grade 4, L: 11 mm), custom-built titanium disc electrodes (Ø 3 mm) or freshly prepared titanium substrates as cathode and a platinized titanium rod (Custom-built, Ø 4.0 mm, L: 10 mm) and Pt coils as anode. Voltage was applied by a laboratory power supply (VoltcraftPlus VSP1410HE) and the circuit inputs were checked before electrolysis with a digital high precision multimeter (Fluke 87 True RMS). Standard conditions were 7 V for 30 s with a current of 300 mA over a junction between the titanium cathode and a platinized titanium anode (cell type "Vert"). The electrolyses in cell types "Hor" and "HorSep" were conducted at 37.0 °C unless stated otherwise. Temperature was controlled by a Julabo FP40 thermostat with a Julabo HD controller. Cell contents were homogenized after electrolysis; 5.00 mL per test were sampled and stored in the dark at 4 °C. Each sampling point was tested three times. The investigated electrolysis times were 10, 20, 30, 60 and 300 s at 300 mA. Open circuit potential as well as current measurements during electrolysis were performed with Pt coil, Pt disc (Ø 3 mm) and Pt wire electrodes and a Vertex Potentiostat (Ivium Technologies) controlled with the IviumSoft software (Version 2.587). Applied cell voltages were 1, 3, 5, 7, 9 and 11 V and the measured potentials were recorded with the subprogram E_{oc} monitor (interval: 0.1 s, run time: 150/300 s, eq, time: 20 s, potential range: 10 V). Electrolysis current was monitored with the subprogram for amperometric detection ($E_{start} = 0.00$ V, interval time: 0.5 s, N samples: 300 pnts, eq. time: 20 s, current range: 1 A).

2.6. Electrochemistry

CV and EIS measurements were performed in custom-built three-electrode electrochemical cell setups mentioned above using a Vertex potentiostat (Ivium Technologies). Data acquisition and evaluation were performed using IviumSoft (Version 2.587). The measurements